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14. ABSTRACT

In this project we plan to analyze androgen- and estrogen-responsive gene expression in the bone marrow. We plan to work to: determine if castration-induced gene expression changes in mouse bone marrow are caused by the deficiency of testosterone or estrogen; analyze androgen- and estrogen-sensitive cytokine and gene expression changes in human bone marrow transplanted into NOD/SCID mice, and; examine androgen- and estrogen-sensitive gene expression in the bone marrow of patients with low and high circulating testosterone levels. Preliminary results show that with the androgen metabolizing genes examined in the bone marrow samples from patients with prostate cancer there is little evidence for a positive correlation between androgen (testosterone) and androgen metabolizing gene expression levels.

15. SUBJECT TERMS
Bone marrow metastasis, androgen, animal model, patient outcome

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"Androgen, Estrogen, and the Bone Marrow Microenvironment"

INTRODUCTION:

In this project we plan to analyze androgen- and estrogen-responsive gene expression in the bone marrow. We postulate that gene and protein expression in the bone marrow microenvironment is subject to regulation by androgen and estrogen and could affect the growth and progression of micrometastatic prostate cancer cells. When prostate cancer cells leave the circulation through fenestrations in the bone vasculature, they lodge in the fertile soil of the bone marrow. Interactions between prostate cancer cells and the bone marrow regulate the early steps of metastasis formation. This environment differs from the environment of established prostate cancer metastasis, in which a fibrotic bone marrow stroma surrounds the cancer cells and cancer cells stimulate an osteoblastic response in adjacent bone. Almost nothing is known about the initial interactions of micrometastatic prostate cancer with the bone marrow microenvironment (BM-ME). During this period critical decisions in the fate of micrometastatic cancer cells occur that determine their latency, survival and proliferation. Most likely, factors in the BM-ME play a major role in regulating the progression of micrometastatic disease. While model systems exist for several steps in metastasis formation, including interactions of prostate cancer cells with endothelial cells and osteoblastic and osteoclastic bone cells, there is no *in-vivo* system to investigate the interactions between prostate cancer cells and the BM-ME. Therefore, there are many unanswered questions related to events that will ultimately determine who develops lethal prostate cancer metastases. In this grant application we will begin to investigate mechanisms that control the fate of prostate cancer cells when they first enter the BM.

Early androgen ablation has a significant survival benefit in patients at risk for prostate cancer recurrence or with increasing PSA levels after surgery or radiation therapy. At the initiation of androgen ablative therapy, the disease is often not apparent by conventional radiographic methods. However, the majority of patients will have micrometastatic disease outside the prostate. Therefore it is important to understand if and how androgen ablative therapy affects the bone marrow cells that surround the micrometastatic cancer cells. In this study, we plan to work to: determine if castration-induced gene expression changes in mouse bone marrow are caused by the deficiency of testosterone or estrogen; analyze androgen- and estrogen-sensitive cytokine and gene expression changes in human bone marrow transplanted into NOD/SCID mice, and; examine androgen- and estrogen-sensitive gene expression in the bone marrow of patients with low and high circulating testosterone levels.

BODY:

This research project was subject to second-level review by the U.S. Army Medical Research Material Command's Human Subjects Research Review Board (HSRRB). Because of this review, study implementation was precluded until we met specific requirements for compliance with human subjects protection and received approval from our local IRB and then the HSRRB. We received final approval from the HSRRB on November 21, 2006.

KEY RESEARCH ACCOMPLISHMENTS:

1) We have obtained human samples of bone marrow from twenty patients with prostate cancer which we have analyzed for testosterone levels.
2) Gene expression has been analyzed for six androgen sensitive genes in four of the twenty bone marrow samples. (androgen sensitive genes: AKR1C1, AKR1C2, AKR1C3, RODH-4, RODH-5, and RL-HSD)
3) We have obtained CD45+ samples from which we are currently analyzing changes in gene expression for androgen sensitive genes.
4) We are organizing the animal work for the human bone marrow xenograft studies in mice.

REPORTABLE OUTCOMES:

A) Testosterone levels for twenty samples from bone marrow of patients with prostate cancer have been analyzed and subdivided into the top and bottom quartiles. (see table)

Table 1. Testosterone levels in patients with prostate cancer

	sample i.d.	BM T - ng/ml
1	23316K	0.2
2	23343R	0.56
3	23334D	0.68
4	23290M	0.69
5	23289C	0.72
6	23333L	0.82
7	23349C	0.82
8	23283K	1.03
9	23329L	1.03
10	23331K	1.03
11	23319B	1.08
12	23300B	1.14
13	23340K	1.25
14	23301H	1.26
15	23285C	1.28
16	23253G	1.31
17	23293M	1.4
18	23291C	1.41
19	23320H	1.66
20	23328J	1.94

Top and bottom quartiles from Table 1

Bone Marrow Testosterone levels		BM T - ng/ml
1	23316K	0.2
2	23343R	0.56
3	23334D	0.68
4	23290M	0.69
5	23289C	0.72
6	23253G	1.31
7	23293M	1.4
8	23291C	1.41
9	23320H	1.66
10	23328J	1.94

low Testosterone Levels

High Testosterone Levels

B) Two bone marrow samples were selected from the top and bottom quartiles for evaluation of five androgen metabolising genes (Table 2).

Table 2. Bone Marrow gene expression levels for selected androgen metabolising genes

Bones Marrow Sample	Testosterone (ng/ml)	AKR1C1	AKR1C2	AKR1C3	RL-HSD	RODH-4
23334D	0.680	0.160	0.062	3.204	0.002	0.006
23343R	0.560	10.056	0.010	0.505	0.026	0.002
23328J	1.940	0.003	0.004	0.039	0.002	0.018
23253G	1.310	7.499	0.317	0.201	0.005	0.034
PEC control	n/a	0.027	0.054	0.867	0.000	0.000

All gene expression values are de-logged Ct's normalized to ACTB

low testosterone | high testosterone

CONCLUSIONS:

Preliminary results show that with the androgen metabolizing genes examined in the bone marrow samples from patients with prostate cancer there is little evidence for a positive correlation between androgen (testosterone) and androgen metabolizing gene expression levels. It is expected that testing the remaining six

samples in the two quartiles will either verify the lack of a relationship between androgen and androgen metabolising gene expression in metastatic prostate cancer of the bone marrow of these samples or reveal a technical problem with the analysis.

REFERENCES:
None